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(71) Applicant(s)

F Hoffmann-La Roche AG
(Incorporated in Switzerland)
124 Grenzacherstrasse, CH-4070 Basle, Switzerland

(72) Inventor(s)

Geo Adam
Philippe Nicolas Huguenin-Virchaux
Vincent Mutel
Heinz Stadler
Thomas Johannes Woltering

(74) Agent and/or Address for Service

Forrester Ketley & Co
Forrester House, 52 Bounds Green Road, LONDON,
N11 2EY, United Kingdom

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C65X C660 C662 C668 C712 C802
U1S S1325 S2417 S2418

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EP 0774455 A1

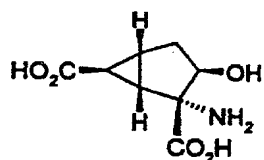
(58) Field of Search

UK CL (Edition Q) C2C CLR
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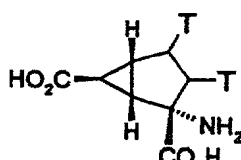
(54) Abstract Title

Novel modulators of metabotropic glutamate receptor function and processes for the preparation thereof

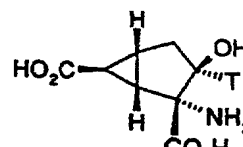
(57) Compound of formulae



I-A

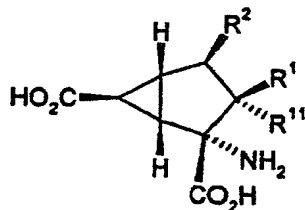


I-B



I-C

and to a novel process for the manufacture of compounds of the general formula



I

wherein

T is tritium;

R¹ is hydroxy, lower alkoxy, lower alkenyloxy, benzyloxy, hydrogen, deuterium or tritium;

R¹¹ is hydrogen, deuterium or optionally tritium, hydroxy or amino and

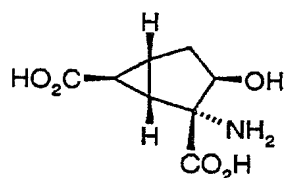
R² is hydrogen or optionally tritium or

R¹ and R² form a bond.

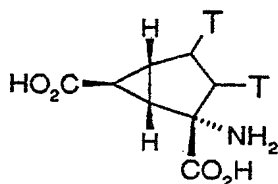
Compounds of general formula I are ligands for the metabotropic glutamate receptors of group II.

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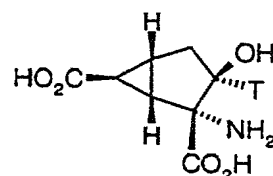
The present invention relates to compounds of formulae



I-A

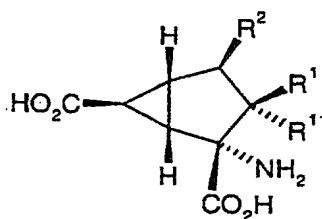


I-B



I-C

and to a novel process for the manufacture of compounds of the general formula



I

5

wherein

T is tritium;

R¹ is hydroxy, lower alkoxy, lower alkenyloxy, benzyloxy, hydrogen, deuterium or tritium;

10 R¹¹ is hydrogen, deuterium or optionally tritium, hydroxy or amino and

R² is hydrogen or optionally tritium or

R¹ and R² form a bond.

Compounds of general formula I are ligands for the metabotropic glutamate receptors of group II.

15 In the central nervous system (CNS) the transmission of stimuli takes place by the interaction of a neurotransmitter, which is sent out by a neuron, with a neuroreceptor. L-glutamic acid, the most commonly occurring neurotransmitter in the CNS, plays a critical role in a large number of physiological processes. The glutamate-dependent stimulus receptors are divided into two main groups. The first main group forms ligand-

controlled ion channels. The metabotropic glutamate receptors (mGluR) belong to the second main group and, furthermore, belong to the family of G-protein-coupled receptors.

At present, eight different members of these mGluRs' are known and of these some even have sup-types. On the basis of structural parameters, the different second messenger signalling pathway and the different affinity to low-molecular weight chemical compounds, these eight receptors can be sub-divided into three sub-groups:

mGluR1 and mGluR5 belong to group I, mGluR2 and mGluR3 belong to group II and mGluR4, mGluR6, mGluR7 and mGluR8 belong to group III.

Generally, these receptors function to modulate the presynaptic release of glutamate, and the postsynaptic sensitivity of the neuronal cell to glutamate excitation. The metabotropic glutamate receptors are useful for the treatment of acute and chronic neurological conditions and psychiatric disorders.

Ligands for the metabotropic glutamate receptors are described, for example, in EP 774 455. Compounds described therein are useful as modulators of metabotropic glutamate receptor function. These compounds are different to compounds of the present formula I by one or two substitutions on the ring molecule. The description of the syntheses for generically encompassed compounds is very uncertain and vague, and it leads to mixtures of diastereomers and/or enantiomers, which would have to be separated into clean isomers by tedious and time-consuming procedures.

It has now surprisingly been found that compounds of the general formula I can be prepared in high yield and in high diastereoisomeric and enantiomeric purity in a new synthetic way.

The following definitions of the general terms used in the present description apply irrespective of whether the terms in question appear alone or in combination.

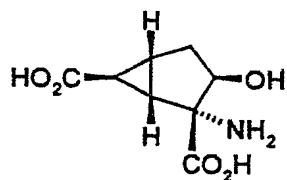
As used herein, the term "lower alkyl" denotes a straight- or branched-chain alkyl group containing from 1-6 carbon atoms, for example, methyl, ethyl, propyl, i-propyl, n-butyl, i-butyl, t-butyl, pentyl or hexyl.

Also, the term "lower alkenyl" denotes the same kind of straight- or branched-chain moiety as described with "lower alkyl", but containing additional multiple bond(s).

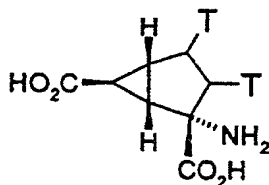
The term "leaving group" means, for example, an iodide-, bromide-, chloride-, methanesulfonate-, tolylsulfonate- or trifluoromethanesulfonate-group.

The term "halogen" denotes chlorine, iodine, fluorine or bromine.

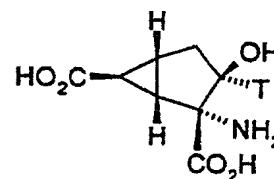
Objects of the present invention are novel compounds of formulae



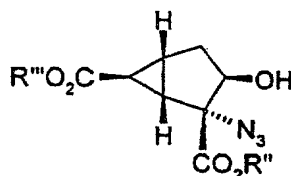
I-A



I-B



I-C



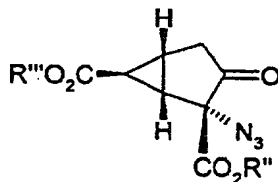
VII

and

per se, wherein compounds of formula VII are used as intermediates for the process for preparation of compounds of formulae I-A and I, in which R'' and R''' are independently from each other benzyl or lower alkyl, racemic mixtures and their corresponding enantiomers of these compounds, the use of compounds of formula I-A for proof of concept studies in animal models of human diseases, the use of compounds of formula I-A for the control or prevention of acute and chronic neurological conditions and psychiatric disorders, and the use of compounds of formulae I-B and I-C as radio ligands in a binding assay in order to screen chemical libraries for new structures, and the process for preparation of compounds of formulae I-A, I-B, I-C and I.

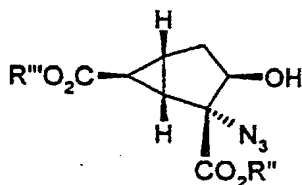
In accordance with the invention the process comprises

a) reducing the carbonyl group of a compound of formula

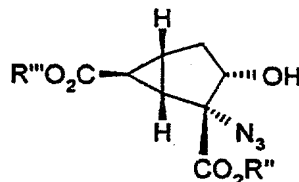


IX

to a hydroxy group of a compound of formula VII and formula X



VII



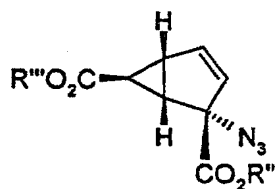
X

and, if desired,

transforming the hydroxy group of a compound of formula VII or X into a leaving group

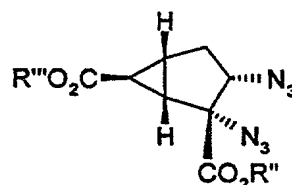
and 1.) eliminating this group to obtain a compound of formula XXVI or

2.) substituting said leaving group with azide to a compound of formula XXVIII



XXVI

or



XXVIII

and if desired,

b) alkylating, alkenylating or benzylating a compound of formula VII

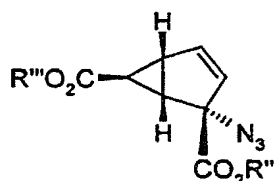
and

5 c) reducing the azide group(s) into (an) amino group(s),

and

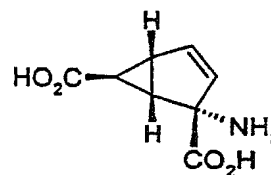
d) hydrolizing the ester groups, and, if desired,

e) hydrogenating the double bond in compounds of formulae



XXVI

or



I-10

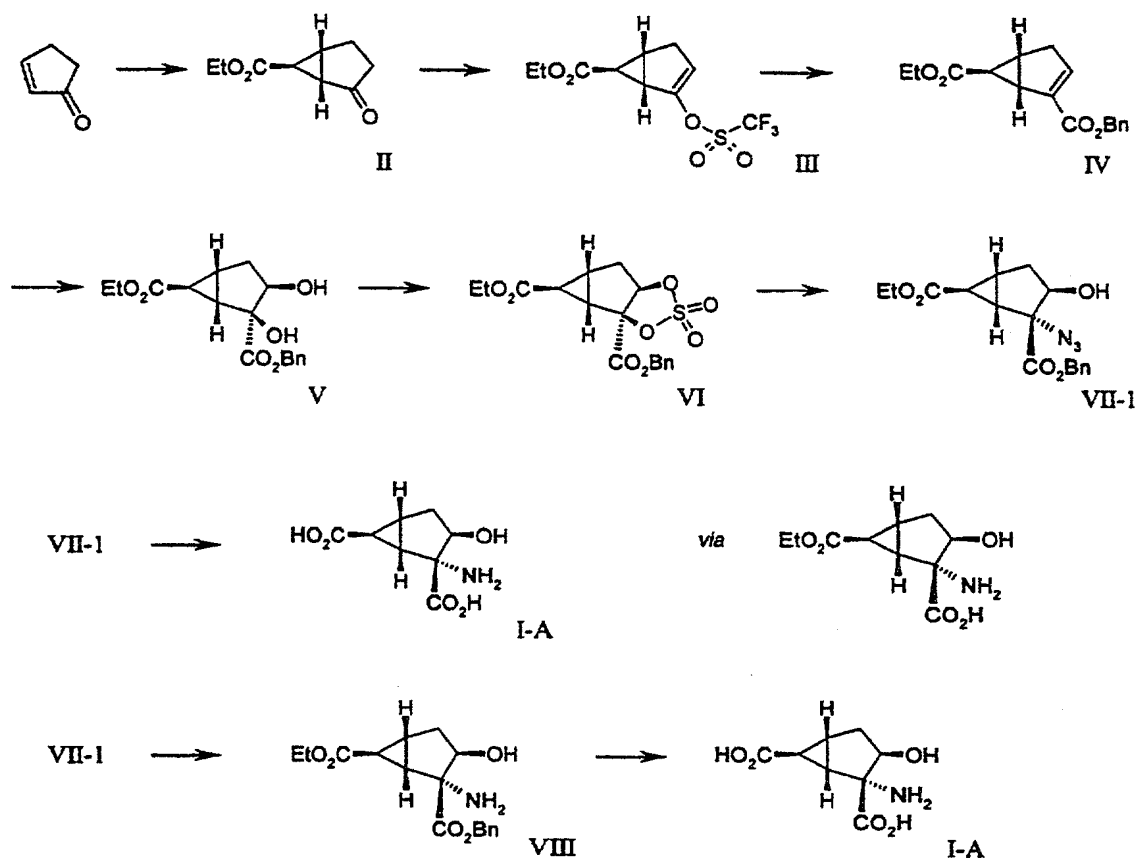
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with tritium-gas.

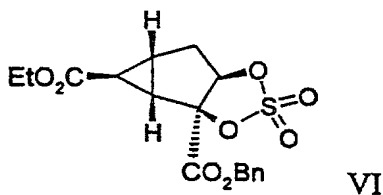
In the following schemes 1-5 are described the processes for preparation of the starting compound of formula VII to compounds of formulae I-A, I-B, and I-C and

15 processes for preparation of compounds of formula I.

Scheme 1



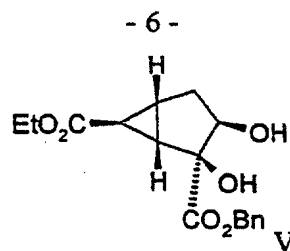
According to scheme 1, compounds of general formula VII can be prepared from compounds of general formula



by reacting with an azide, preferably sodium azide in a polar, aprotic solvent, like for example DMF, at temperatures of around 100° C or in aqueous solutions of water-miscible solvents, like for example acetone or tetrahydrofuran, at temperatures around 50° C and then hydrolizing the formed sulfate half ester with an acid, preferably sulfuric acid.

- 10 An example for this kind of transformation can be found in *J. Am. Chem. Soc.* 1988, 110 (22), 7538.

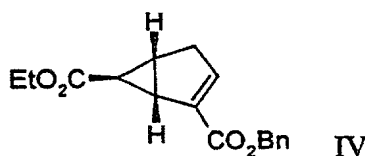
Compounds of general formula VI can be prepared by reacting compounds of general formula V



with thionyl chloride in an aprotic solvent to yield a cyclic sulfite. If desired, the reaction can be carried out in the presence of a base. Then the cyclic sulfite is oxidized to the cyclic sulfate. Suitable solvents for the formation of the sulfite are, for example, chlorinated solvents such as dichloromethane, chloroform, carbon tetrachloride or dichloroethane. Suitable bases can be selected from amines, for example, but not limited to, triethylamine or diisopropylethylamine. A preferred oxidant is, for example sodium-periodate in the presence of catalytic amounts of a ruthenium salt, such as ruthenium trichloride. The oxidation is preferably carried out in a solvent mixture consisting of carbon tetrachloride, acetonitrile and water in a ratio of 2 to 2 to 3.

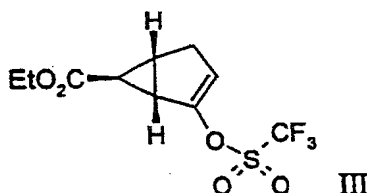
A procedure for the preparation of cyclic sulfates can be found in US 5321143.

Compounds of general formula V can be prepared by performing an asymmetric *cis*-hydroxylation (Sharpless AD) reaction on compounds of general formula



This reaction allows for the selective preparation of one desired isomer of compounds of general formula V out of four possible isomers (two diastereoisomeric pairs of enantiomers). Examples for the use of the asymmetric *cis*-hydroxylation reaction can be found in for example *J. Org. Chem.* 1996, 61(8), 2582-2583 and *Tetrahedron:Asymmetry* 1993, 4(1), 133-141.

Compounds of general formula IV can be prepared from compounds of general formula



by a transition metal catalyzed carbon monoxide insertion in the presence of the alcohol R'''-OH.

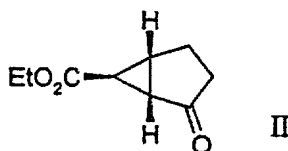
wherein R''' is benzyl or lower alkyl,

The reaction can be carried out in R'''-OH as the solvent or aprotic solvents such as, for example tetrahydrofuran or DMF with stoichiometric amounts (one to two equivalents) of R'''-OH present. The transition metal preferred is palladium in the form of its salt, like for example palladium(II)-acetat or palladium(0)-compounds, for example,

5 tetrakis(triphenylphosphine)palladium.

Examples for such kind of CO-insertions can be found in *J. Org. Chem.* 1992, 57, 5979.

Compounds of general formula III can be prepared by methods known in the art by reacting a carbonyl compound of general formula



10 with a base in the presence of N-phenyl-bis(trifluoromethanesulfonimide) or trifluoromethanesulfonic acid anhydride. The base can be selected from sterically hindered amines, for example 2,6-di-*tert*.-butyl pyridine or from amides, such as lithium diisopropyl amide. The reaction can be carried out at temperatures between -78° and +20° C. In the case of amine bases, the solvent can be selected from the class of chlorinated solvents, such as, for example, dichloromethane, chloroform or 1,2-dichloroethane. In the case of the amide bases, the solvent can be selected from the class of ethers, for example diethyl ether or tetrahydrofuran.

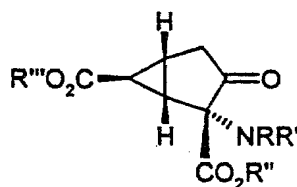
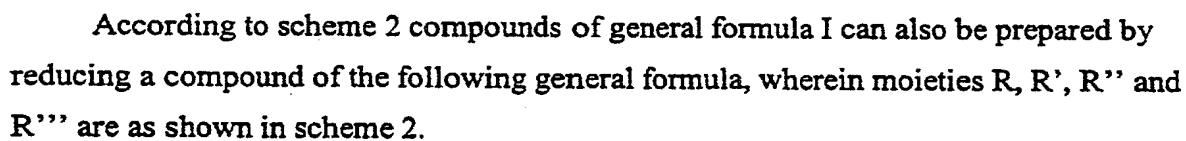
A general method for the preparation of vinyl-trifluoromethanesulfonates can be found in *J. Org. Chem.* 1992, 57, 5979.

20 According to the present invention, compounds of general formula I in which R² is hydrogen and R¹ and R¹¹ have the meaning as described above, can be prepared by reducing the azide group in compounds of general formula VII to the amino group to obtain compounds of general formula VIII and hydrolizing the ester groups in compounds of general formula VIII to the carboxylic acids. It is also possible, to cleave the esters first to the carboxylic acids to obtain compounds of general formula XI (*cf.* scheme 2) and then reduce the azide group to the amino group. A practical method for the reduction of azides to amines is for example the catalytic hydrogenation in the presence of a transition metal catalyst, like for example nickel, platinum or palladium (see for example *Org. Syntheses*, Coll. Vol. V, 1973, 586). Another suitable method is the Staudinger-type reduction of azides using phosphines, like for example triphenyl phosphine in aqueous solutions of an ether, like for example tetrahydrofuran, as described in *J. Chem. Soc. Chem. Comm.* 1986, 409. Still another method for the reduction of azides to amines is the treatment with metal

hydrides, like for example lithium aminoborohydride, as shown in: ACS Symp. Ser. 1996, 641 (Reductions in Organic Synthesis), 153-166), or sodium borohydride in the presence of a transition metal salt, like for example nickel(II)chloride hexahydrate, as shown in *Chem. Ind. (London)* 1987, 764.

- 5 The cleavage of the ester groups can be achieved by using methods known to people skilled in the art, for example, by treatment of the starting ester with aqueous acid or base at ambient or elevated temperatures, or by the cleavage of esters sensitive to catalytic hydrogenation (for example, benzyl esters and the like) using hydrogen in the presence of a transition metal catalyst like for example nickel, platinum or palladium.

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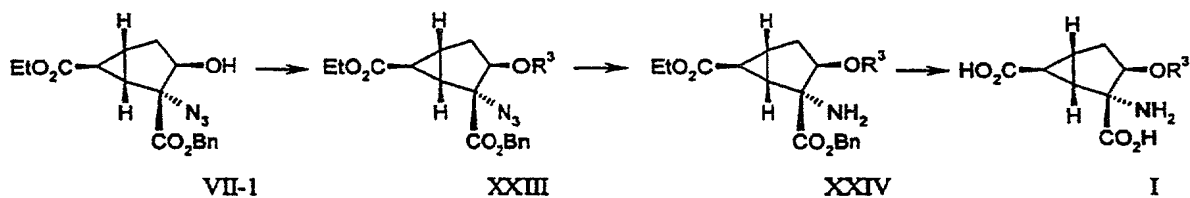
with a reducing agent, for example with sodium-borohydride, lithium-borohydride, sodium-borodeuteride, lithium-borodeuteride, sodium-borotritiide and lithium-trimethoxyborotritiide in the presence or absence of cerium trichloride. The reaction is carried out in solvent mixtures of a protic solvent, for example an alcohol, such as methanol or ethanol, and an ether, such as diethylether or tetrahydrofuran, at temperatures between -78° and +30° C. The variation of the solvent composition, the temperature, the reducing agent, the use of cerium trichloride and the transformation of the azide into an N-acylamino group prior to reduction of the ketone, like for example the shown reaction of IX to XVI by treatment with thioacetic acid as described in *J. Org. Chem.* 1988, 53, 1581, allows the one skilled in the art to obtain the desired diastereoisomer of compounds of general formula I, in which R¹ represents hydroxy, R¹¹ represents hydrogen, deuterium or tritium and R² represents hydrogen.

A comprehensive overview for the use of reducing agents can be found in Larock, *Comprehensive Organic Transformations*, Verlag Chemie 1989, 527.

Compounds of general formula IX can be prepared by oxidizing a compound of general formula VII. The reaction is carried out in a chlorinated solvent, for example in dichloromethane, chloroform or dichloroethane. Suitable oxidizing agents are, for example derivatives of chromic acid, such as PDC or PCC, or hypervalent iodine-compounds, such as IBX or the Dess-Martin-reagent. The reaction is preferably carried out at temperatures between -20° and +30° C under an inert atmosphere.

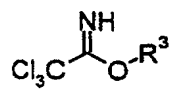
A comprehensive overview for oxidations using the reagents described above can be found in R.C. Larock, *Comprehensive Organic Transformations*, Verlage Chemie 1989, 604.

Scheme 3



According to scheme 3 the O-alkylated compounds of general formula I, wherein R³ is lower alkyl (I-7 and I-8), lower alkenyl (I-9) or benzyl (I-10) can be prepared from compounds of the general formulae XXIII (XXIII-1 with R³ = CH₃; XXIII-2 with R³ = allyl; and XXIII-3 with R³ = benzyl;) and XXIV (XXIV-1 with R³ = CH₃; XXIV-2 with R³ = allyl; and XXIV-3 with R³ = benzyl;) by the methods described above for the preparation of compound I-A.

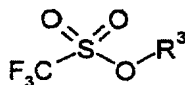
Compounds of general formula XXIII in which R³ represents lower alkyl, lower alkenyl or benzyl can be prepared by reacting a compound of general formula VII-1 with a trichloro-acetimide of general formula



- 5 in which R³ represents lower alkyl, lower alkenyl or benzyl in an aprotic solvent in the presence of an acid.

The acid, which is preferably used in catalytic amounts, can be a Bronsted-acid such as trifluoromethane sulfonic acid or trifluoroacetic acid or a Lewis-acid such as trimethylsilyl-trifluoromethane sulfonate or borontrifluoride-etherate. Suitable solvents are preferably aprotic solvents like ethers, such as diethylether or tetrahydrofuran, chlorinated solvents, such as for example dichloromethane, chloroform or dichloroethane, or mixtures of chlorinated solvents and hydrocarbons, such as hexane or cyclohexane. The reaction can be carried out at temperatures between -50° and +40° C under an inert atmosphere.

- 10 Another way to prepare the compounds of general formula VII in which R¹ represents lower alkyl, lower alkenyl or benzyl is the treatment of compounds of general formula VII with a trifluoromethanesulfonate of general formula



in which R³ has the meaning as described above, in a suitable solvent and in the presence of a base.

- 20 The base can be selected from the group of sterically hindered amines like, for example, but not limited to, 2,6-di-*tert.*-butyl-pyridine. The reaction is preferably carried out in aprotic solvents, for example ethers, such as diethylether or tetrahydrofuran, chlorinated solvents, for example dichloromethane, chloroform or dichloroethane or mixtures of chlorinated solvents or hydrocarbons, for example hexane or cyclohexane. The reaction is preferably carried out at temperatures between -50° and +50° C under an inert atmosphere.

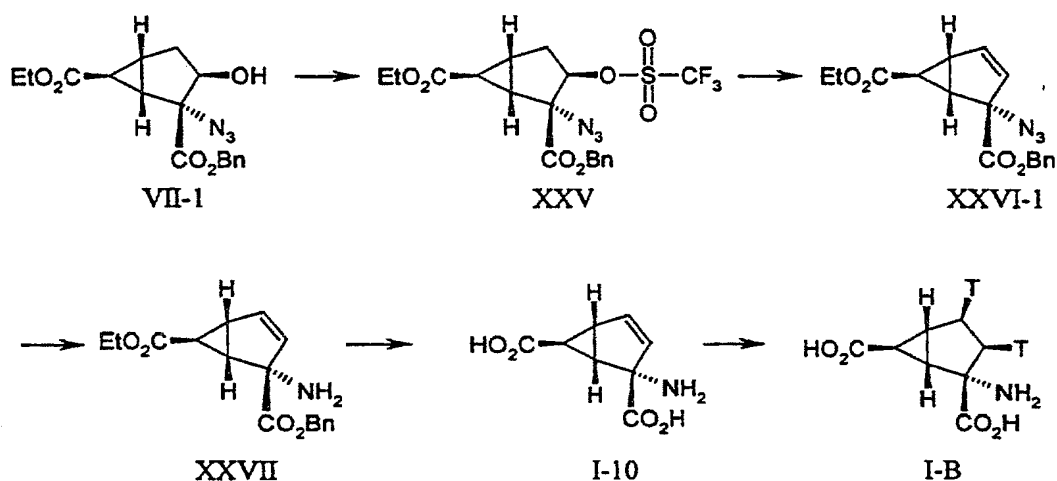
Still another way to prepare compounds of general formula XXIII in which R³ represents lower alkyl, lower alkenyl or benzyl is the treatment of compounds of general formula VII with electrophilic agents of general formula

- 30 R^3-X

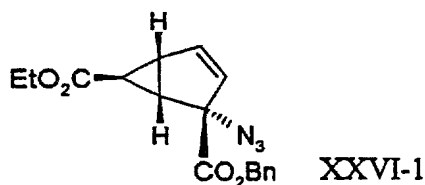
in which R³ has the meaning as described above and X represents a leaving group, for example iodide, bromide, methanesulfonate and tolylsulfonate, in a suitable solvent in the

presence of a base. The reaction is preferably carried out in polar, aprotic solvents, for example chlorinated solvents such as dichloromethane, chloroform or dichloroethane, or amides, for example, dimethylformamide and N-methyl-pyrrolidinone. The base can be selected from the group of sterically hindered amines, for example, but not limited to, 2,6-di-*tert.*-butyl-pyridine or from the hydride-type such as, for example, sodium hydride or potassium hydride. Other possible bases can be selected from the group of amides, such as for example, sodium hexamethyl disilazide or lithium diisopropylamide. The reaction is preferably carried out at temperatures between -50° and +50° C under an inert atmosphere.

Scheme 4

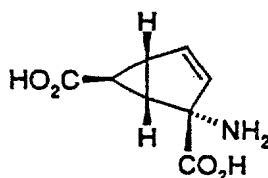


According to scheme 4 compounds of general formula I, in which R¹ represents hydrogen and R¹¹ and R² represent tritium can be prepared by treating a compound of general formula



with a mixture of hydrogen and tritium gas in the presence of a transition metal. If desired, the ester groups may be transformed to the carboxylates prior to the concomitant reduction of the azide group.

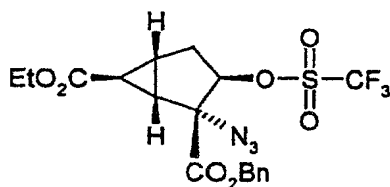
Compounds of general structure



I-10

can be prepared by reducing the azide group in compounds of general formula XXVI using the reagents and conditions as described above, preferably with trimethylphosphine as the reducing agent. The reduction is preferably carried out under the conditions as described above for the use of triphenylphosphine. Then the ester groups can be transformed into carboxylic acids by the methods as described above. Again, if desired, the ester groups may be transformed to the carboxylates prior to the reduction of the azide group.

Compounds of general formula XXVII can be prepared by eliminating the anion of trifluoromethane sulfonic acid from a compound of general formula



XXV

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in the presence of a base.

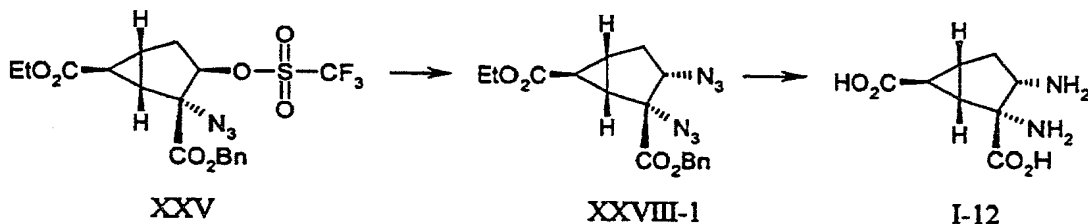
The reaction can be carried out in aprotic solvents, for example ethers, such as diethyl ether or tetrahydrofuran at temperatures between 20° and 35° C (diethylether), respectively 20° and 50° C (tetrahydrofuran). The base may be selected from the amine bases, like for example DBU and the like.

15

Compounds of general formula XXV may be prepared by converting compounds of general formula VII into their trifluoromethanesulfonyl esters. The reaction is a standard reaction in organic chemistry and can be carried out as described in Larock, *Comprehensive Organic Transformations*, Verlag Chemie 1989, 360.

20

Scheme 5

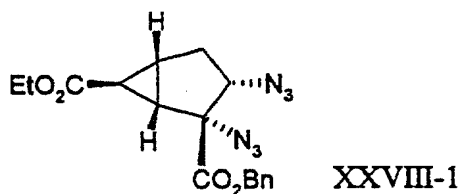


XXV

XXVIII-1

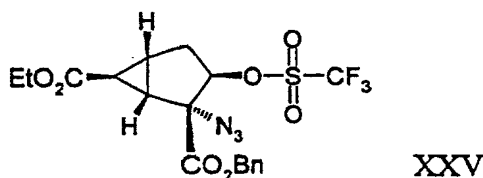
I-12

According to scheme 5 compounds of general formula I, in which R¹ and R² represent hydrogen and R¹¹ represents amino can be prepared from compounds of general formula



by reduction of the azide groups using the reagents and conditions as described above. Then the ester groups can be transformed into carboxylic acids by the methods as described above. If desired, the ester groups may be transformed to the carboxylates prior to the reduction of the azide groups.

Compounds of general formula XXVIII can be prepared by substituting the trifluoromethyl sulfonyl group in compounds of general formula



with an azide, preferably sodium azide, in a polar, aprotic solvent like for example DMF at temperatures of around 60° to 100° C.

Compounds of formula I-A may be used in the control or prevention of acute and chronic neurological conditions and psychiatric disorders. In "Mol. Pharmacology, Vol.53, 228-233, (1998)" it is described the in vitro binding of a selective group II metabotropic glutamate receptor radioligand, [3H]LY354740 in rat brain. The inhibition by LY354740 was antagonised with the group II selective competitive antagonist of the compound of formula I-A (pKB= 6.0; Ki mGluR2 = 0.052 μM; Ki mGluR3 = 0.089 μM).

The following Examples illustrate the present invention without limiting it. All temperatures are given in degrees Celsius.

Example 1

(1RS,5SR,6RS)-2-Trifluoromethanesulfonyloxy-bicyclo[3.1.0]hex-2-ene-carboxylic acid ethyl ester (III)

To a solution of diisopropylamine (11.94 mL, 84.6 mmol) in THF (77 mL) was added dropwise n-BuLi (47.6 mL, 76.1 mmol, 1.6 M solution in hexane) at 0° C and stirred for 10 min at 0° C. After cooling to -78° C a solution of (1S,5R,6S)-2-oxo-bicyclo[3.1.0]hexane-6-carboxylic acid ethyl ester (II) (11.86 g, 70.5 mmol) in THF (39 mL) was added dropwise within 25 min. Stirring was continued at -78° C for 1 h,

whereupon a solution of N-phenyl-bis(trifluoromethylsulfonyl)imine (27.7 g, 77.5 mmol) in THF (83 mL) was added and then stirred for 90 min at 23° C. Aqueous workup with ether, sat. NaHCO₃-sol., brine, drying over Na₂SO₄, removal of the solvent under vacuum left an orange-brown oil, which was purified by silica gel column chromatography with
 5 hexane/ethyl acetate 9:1 to give (1RS,5SR,6RS)-2-trifluoromethanesulfonyloxy-bicyclo[3.1.0]hex-2-ene-carboxylic acid ethyl ester (III) (18.47 g, 87%) as a slightly brown oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.27 (3H, t, J = 7.1 Hz), 1.46 (1H, m), 2.28 (1H, m), 2.55 (2H, m), 2.79 (1H, m), 4.15 (2H, q, J = 7.1 Hz), 5.41 (1H, m); MS[EI] 300 (M⁺).

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Example 2

(1RS,5SR,6RS)-Bicyclo[3.1.0]hex-2-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (IV)

A solution of (1RS,5SR,6RS)-2-trifluoromethanesulfonyloxy-bicyclo[3.1.0]hex-2-ene-carboxylic acid ethyl ester (III) (14.48 g, 48.2 mmol), Pd(OAc)₂ (326 mg, 1.45 mmol),
 15 PPh₃ (760 mg, 2.9 mmol), benzyl alcohol (10.0 mL, 96.5 mmol) and Et₃N (13.5 mL, 96.5 mmol) in DMF (195 mL) was purged with CO for 10 min and then stirred for 5 h at 23° C under a balloon with CO. Aqueous workup with ether, 1 N HCl-sol., sat. NaHCO₃-sol. and brine was followed by drying over MgSO₄. Removal of the solvent under vacuum left a dark brown oil, which was purified by silica gel column chromatography with
 20 hexane/ethyl acetate 9:1 to yield (1RS,5SR,6RS)-bicyclo[3.1.0]hex-2-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (IV) (10.36 g, 75%) as a yellow oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.13 (1H, m), 1.26 (3H, t, J = 7.1 Hz), 2.25 (1H, m), 2.64 (1H, m), 2.79-2.91 (2H, m), 4.10 (2H, q, J = 7.1 Hz), 5.18 (1H, d, J = 12 Hz), 5.24 (1H, d, J = 12 Hz), 6.58 (1H, bs), 7.30-7.40 (5H, m); MS[EI] 286 (M⁺).

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Example 3

(1S,2S,3R,6S)-2,3-Dihydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (V)

A solution of (1RS,5SR,6RS)-bicyclo[3.1.0]hex-2-ene-2,6-dicarboxylic acid 2-benzyl
 30 ester 6-ethyl ester (IV) (11.69 g, 40.4 mmol), K₂[OsO₂(OH)₄] (99 mg, 0.27 mmol), (DHQD)₂PHAL (1.05 g, 1.35 mmol), K₃Fe(CN)₆ (26.6 g, 80.8 mmol), K₂CO₃ (11.2 g, 80.8 mmol) and MeSO₂NH₂ (11.53 g, 121.2 mmol) in *tert.*-butanol (140 mL) and H₂O (140 mL) was stirred vigorously at 4° C for 24 h. After addition of Na₂SO₃ (40.4 g) and stirring for 30 min at 23° C the mixture was diluted with water (300 mL) and extracted with ethyl
 35 acetate (3 × 300 mL). The combined organic layers were washed with 2 N NaOH-sol. (200

mL) and brine (200 mL) followed by drying over Na₂SO₄. Removal of the solvent under vacuum left a dark brown solid (13.51 g), which was subjected to silica gel column chromatography with hexane/ethyl acetate 2:1->3:2->1:1 to yield the residual starting material as a yellow oil (3.58 g, 31%, 65%ee), the undesired diastereomeric diol as a yellow oil (1.30 g, 10%) and the crystalline diol (1S,2S,3R,6S)-2,3-dihydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (5.91 g, 45.7%, 63%ee) as a pale yellow solid. The latter material was twice recrystallized from ethyl acetate/ether/hexane to give enantiopure (1S,2S,3R,6S)-2,3-dihydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (V) (3.36 g, 26%, >99%ee) as white needles. ¹H-NMR (250 MHz, CDCl₃) δ 1.24 (3H, t, *J* = 7.1 Hz), 1.80-1.92 (2H, m), 1.99 (1H, m), 2.08 (1H, dd, *J* = 6.9, 2.9 Hz), 2.30-2.38 (2H, m), 3.82 (1H, s), 4.10 (2H, q, *J* = 7.1 Hz), 5.20 (1H, d, *J* = 12.3 Hz), 5.34 (1H, d, *J* = 12.3 Hz), 7.36 (5H, bs); MS[ISP] 321 (M+H⁺); mp 112-114° C; α_D²⁰ -73.35° (c = 1.17, CHCl₃).

Example 4

(1S,1aS,1bS,4aR,5aR)-3,3-Dioxo-tetrahydro-2,4-dioxo-6-thia-cyclopropa[a]pentalene-1,1b-dicarboxylic acid 1b-benzyl ester 1-ethyl ester (VI)

To a solution of (1S,2S,3R,6S)-2,3-dihydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (V) (2.66 g, 8.32 mmol) in CH₂Cl₂ (14 mL) at 0° C was added SOCl₂ (1.21 mL, 16.64 mmol) and stirring was continued at 40° C until tlc indicated complete conversion to the cyclic sulfite. The solvent and excess SOCl₂ were removed under vacuum, the residual oil was dissolved in CCl₄ (8.3 mL), CH₃CN (8.3 mL) and H₂O (12.5 mL) and cooled to 0° C. NaIO₄ (2.67 g, 12.5 mmol) and RuCl₃ hydrate (33 mg) were added and the mixture was stirred at 23° C for 30 min. Aqueous workup with ether, water and brine was followed stirring of the organic phase with MgSO₄ and a spatula tip of activated carbon. After filtration through celite the solvent was removed under vacuum to yield a pale the crude cyclic sulfate as a brown oil (3.31 g). An analytical sample was obtained by silica gel column chromatography with hexane/ethyl acetate 2:1 to yield (1S,1aS,1bS,4aR,5aR)-3,3-dioxo-tetrahydro-2,4-dioxo-6-thia-cyclopropa[a]pentalene-1,1b-dicarboxylic acid 1b-benzyl ester 1-ethyl ester (VI) (98%). ¹H-NMR (250 MHz, CDCl₃) δ 1.25 (3H, t, *J* = 7.2 Hz), 1.67 (1H, t, *J* = 3.5 Hz), 2.28 (1H, m), 2.52-2.62 (3H, m), 4.11 (2H, m), 5.32 (2H, s), 7.38 (5H, s); MS[ISP] 400 (M+NH₄⁺); α_D²⁰ -36.08° (c = 1.13, CHCl₃).

Example 5

(1S,2R,3R,5R,6S)-2-Azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII-1)

The crude cyclic sulfate VI was dissolved in acetone (45 mL) and H₂O (4.5 mL), NaN₃ (720 mg, 11.1 mmol) was added and the mixture was stirred at 50° C until tlc indicated complete conversion of the cyclic sulfate. The solvent was removed under vacuum, the residue partitioned between ether (160 mL) and water (4.5 mL), cooled to 0° C, whereupon 20% H₂SO₄ (13.5 mL) was dropwise added. The mixture was stirred vigorously at 23° C for 37 h, the layers were separated, the organic layer washed with sat. NaHCO₃-sol. and brine, dried over MgSO₄. After removal of the solvent under vacuum, the residual oil (2.78 g, 97%) was purified by silica gel column chromatography with hexane/ethyl acetate 9:1->5:1 to give (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII-1) (1.79 g, 62%) as a colorless oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.26 (3H, t, J = 7.1 Hz), 1.81 (1H, t, J = 3.1 Hz), 2.04-2.20 (3H, m), 2.25 (1H, dd, J = 6.9, 2.9 Hz), 2.34 (1H, dd, J = 7.7, 3.9 Hz), 3.80 (1H, bq, J = 9 Hz), 4.12 (2H, q, J = 7.1 Hz), 5.27 (1H, d, J = 12.2 Hz), 5.34 (1H, d, J = 12.2 Hz), 7.36-7.40 (5H, m); MS[ISN] 404 (M+OAc⁻); α_D²⁰ -48.43° (c = 1.09, CHCl₃).

Example 6

(1S,2R,3R,5R,6S)-2-Amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-A)

A solution of (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII-1) (1.55 g, 4.49 mmol) in HOAc (20 mL) and H₂O (5 mL) was hydrogenated in the presence of Pd/C (100 mg, 10% Pd/C) at 23° C for 18 h. The catalyst was removed by filtration, the filter cake washed with 50% aqueous acetic acid. After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (55 mL) for 4 h. The solution was cooled to 23° C, filtered, washed with water and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (45 mL) and propylene oxide (24 mL) and refluxed for 15 min, whereupon the amino acid precipitated. After cooling to 23° C, the product was filtered off, washed with ether and dried to give (1S,2R,3R,5R,6S)-2-amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-A) (784 mg, 87%) as a white solid. ¹H-NMR (250 MHz, D₂O) δ 1.87

(1H, t, $J = 3.1$ Hz), 2.15-2.26 (3H, m), 2.38 (1H, dd, $J = 13.0, 7.6$ Hz), 3.97 (1H, dd, $J = 8.6, 7.4$ Hz); MS[ISP] 202 ($M+H^+$); mp $>250^\circ$ C; $\alpha_D^{20} +7.41^\circ$ ($c = 1.01, H_2O$).

Example 7

5 (1S,2R,3R,5R,6S)-2-Amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VIII)

To a solution of (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII-1) (200 mg, 0.58 mmol) in MeOH (5.8 mL) and a few drops of chloroform at 0° C was added $NiCl_2 \cdot 6 H_2O$ (661 mg, 2.78 mmol) was dissolved in acetone (45 mL) and H_2O (4.5 mL), NaN_3 (720 mg, 11.1 mmol) and stirred for 10 min at 0° C. Upon cautious addition of $NaBH_4$ (175 mg, 4.62 mmol) the reaction mixture turned immediately black, stirring was continued at 0° C for 10 min and then hydrolyzed with water and ether. After additional stirring for 10 min at 0° C, the reaction mixture was extracted with ether, washed with brine (2×50 mL) and dried over Na_2SO_4 and filtered through celite. The solvent was removed under vacuum, the residue was purified by silica gel column chromatography with ethyl acetate/MeOH 95:5 (+ 0.6% Et_3N) to give (1S,2R,3R,5R,6S)-2-amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VIII) (106 mg, 57%) as a yellow oil. 1H -NMR (250 MHz, $CDCl_3$) δ 1.25 (3H, t, $J = 7.1$ Hz), 1.75 (1H, t, $J = 3.5$ Hz), 1.97 (1H, m), 2.12 (1H, dd, $J = 7, 3.5$ Hz), 2.14-2.28 (4H, m), 2.33 (1H, dd, $J = 12.5, 7$ Hz), 3.65 (1H, dd, $J = 8, 7$ Hz), 4.10 (2H, q, $J = 7.1$ Hz), 5.22 (1H, d, $J = 12.5$ Hz), 5.27 (1H, d, $J = 12.5$ Hz), 7.38 (5H, m); MS[ISP] 320 ($M+H^+$); $\alpha_D^{20} -0.56^\circ$ ($c = 0.89, CHCl_3$).

Example 8

25 (1S,2R,5R,6S)-2-Azido-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (IX-1)

To a solution of (1S,2R,3R,5R,6S)-2-Azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII-1) (960 mg, 2.78 mmol) in DCM (18 mL) was added PCC (2.40 g, 50% on silica gel) at 0° C and stirring was continued at 23° C for 20 h. The reaction mixture was placed on a silica gel column and the product was eluted with DCM to yield (1S,2R,5R,6S)-2-azido-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (IX-1) (746 mg, 78%) as a white solid. 1H -NMR (250 MHz, $CDCl_3$) δ 1.27 (3H, t, $J = 7.2$ Hz), 1.62 (1H, t, $J = 3.4$ Hz), 2.29 (1H, m), 2.44 (1H, dd, $J = 7.7, 3.1$ Hz), 2.55 (1H, d, $J = 19.2$ Hz), 2.98 (1H, dd, $J = 19.2, 5.6$ Hz),

4.13 (2H, q, $J = 7.2$ Hz), 5.29 (2H, s), 7.36-7.40 (5H, m); MS[ISP] 361 ($M+NH_4^+$); mp 46-48° C; $\alpha_D^{20} +210.91^\circ$ ($c = 1.07$, $CHCl_3$).

Example 9

5 (1S,2R,3S,5R,6S)-2-Azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (X-1)

To a solution of (1S,2R,5R,6S)-2-azido-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (IX-1) (100 mg, 0.29 mmol) in EtOH (1.7 mL) and THF (0.5 mL) was added $NaBH_4$ (22 mg, 0.58 mmol) at -50° C and stirring was continued at -50° C
10 for 4 h. The reaction mixture was poured on ice, acidified with 1 N HCl and extracted with ether. After washing with sat. $NaHCO_3$ -sol., brine and drying over $MgSO_4$, the crude product was purified by silica gel column chromatography with hexane/EtOAc 5:1 to give (1S,2R,3S,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (X-1) (51 mg, 51%) as a colorless oil. 1H -NMR (250 MHz,
15 $CDCl_3$) δ 1.26 (3H, t, $J = 7.2$ Hz), 2.03-2.18 (3H, m), 2.31-2.48 (2H, m), 2.53 (1H, t, $J = 4$ Hz), 4.13 (2H, q, $J = 7.2$ Hz), 4.21 (1H, bs), 5.26 (2H, s), 7.36-7.40 (5H, m); MS[EI] 300 [$(M-OEt)^+$];

Example 10

20 (1S,2R,3S,5R,6S)-2-Amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-2)

A solution of (1S,2R,3S,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (X-1) (50 mg, 0.145 mmol) in HOAc (4 mL) and H_2O (1 mL) was hydrogenated in the presence of Pd/C (11 mg, 10% Pd/C) at 23° C for 23 h. The catalyst was removed by filtration, the filter cake washed with 50% aqueous acetic acid.
25 After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (6.75 mL) for 4 h. The solution was cooled to 23° C, filtered, washed with water and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (5 mL) and propylene oxide (2 mL) and refluxed for 15 min, whereupon the amino acid precipitated. After cooling to 23° C, the product was filtered off, washed with ether and dried to give
30 (1S,2R,3S,5R,6S)-2-amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-2) (24 mg, 86%) as a white solid. 1H -NMR (250 MHz, D_2O) δ 2.05 (1H, d, $J = 15$ Hz), 2.10-2.20 (2H, m), 2.12 (1H, m), 2.61 (1H, m), 4.20 (1H, d, $J = 7.2$ Hz); MS[ISP] 202 ($M+H^+$); mp 208° C (dec.); $\alpha_D^{20} +27.52^\circ$ ($c = 1.03$, H_2O).

Example 11

(1S,2R,3R,5R,6S)-2-Azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (XI)

To a solution of (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII) (845 mg, 2.45 mmol) in THF (21 mL) and H₂O (6 mL) was added LiOH•H₂O (411 mg, 9.8 mmol) and the mixture was stirred at 23° C for 18 h. The benzyl alcohol was extracted with ether, the aqueous layer acidified with 1M KHSO₄-sol., saturated with solid NaCl, extracted with EtOAc (4 × 50 mL) and dried over MgSO₄. After removal of the solvent under vacuum crude (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (XI) (581 mg, quant.) was obtained as white solid, sufficiently pure for further transformations. ¹H-NMR (250 MHz, DMSO) δ 1.75 (1H, t, J = 3.5 Hz), 1.84 (1H, m), 1.92-2.07 (2H, m), 2.15 (1H, dd, J = 12.5, 7 Hz), 3.87 (1H, bt J = 7 Hz); MS[ISN] 226 [(M-H)⁻]; mp 124-128° C; α_D²⁰ -61.48° (c = 0.90, H₂O).

Example 12

(1S,2R,3R,5R,6S)-2-Azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid dimethyl ester (XII)

Crude (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (XI) (515 mg, 2.27 mmol) was dissolved in MeOH (20 mL) and stirred in the presence of conc. H₂SO₄ (0.1 mL) at 23° C for 7 d. The mixture was diluted with ether, washed with sat. NaHCO₃-sol., brine and dried over MgSO₄. After removal of the solvent in vacuum, the residue was purified by silica gel column chromatography with hexane/ethyl acetate 2:1 to give (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid dimethyl ester (XII) (500 mg, 86%) as a yellow oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.84 (1H, t, J = 3.1 Hz), 2.04-2.26 (2H, m), 2.25 (1H, dd, J = 6.9, 3.0 Hz), 2.37 (1H, dd, J = 12.5, 7.8 Hz), 3.69 (3H, s), 3.83 (1H, bq, J = 9 Hz), 3.90 (3H, s); MS[ISP] 273 (M+NH₄⁺); α_D²⁰ -53.33° (c = 1.11, CHCl₃).

Example 13

(1S,2R,5R,6S)-2-Azido-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic dimethyl ester (XIII)

To a solution of (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid dimethyl ester (XII) (239 mg, 0.94 mmol) in DCM (6 mL) was added PCC (1.13 g, 2.62 mmol, 50% on silica gel) at 0° C and the mixture was stirred at 23° C

for 2 d. The PCC was removed by filtration through a silica gel column and the pure (1S,2R,5R,6S)-2-azido-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic dimethyl ester (XIII) was obtained by silica gel column chromatography with hexane/EtOAc 2:1 as a white solid (152 mg, 64%). ¹H-NMR (250 MHz, CDCl₃) δ 1.64 (1H, t, *J* = 3.4 Hz), 2.34 (1H, ddd, *J* = 7.7, 5.5, 3.2 Hz), 2.46 (1H, dd, *J* = 7.7, 3.1 Hz), 2.56 (1H, d, *J* = 19.2 Hz), 3.06 (1H, dd, *J* = 19.2, 5.5 Hz), 3.71 (3H, s), 3.89 (3H, s); MS[EI] 222 [(M-OCH₃)⁺]; mp 62-65° C;

Example 14

10 (1S,2R,3R,5R,6S)-2-Azido-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid dimethyl ester (XIV) and (1S,2R,3S,5R,6S)-2-Azido-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid dimethyl ester (XV)

A suspension of (1S,2R,5R,6S)-2-azido-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic dimethyl ester (XIII) (123 mg, 0.486 mmol) and anhydrous CeCl₃ (120 mg, 0.486 mmol) in MeOH (3.8 mL) was sonicated at 23° C for 2 min and cooled to -78° C. NaBD₄ (21 mg, 0.486 mmol) was added in one portion and the mixture was stirred at -50° C for 30 min. The reaction was quenched by addition of HOAc (ca. 0.5 mL), warming up to 23° C and stirring for 10 min. After dilution with ether and extraction with sat. NaHCO₃-sol., brine and drying over MgSO₄, the crude product was purified by silica gel column chromatography with hexane/EtOAc 2:1 to give (1S,2R,3S,5R,6S)-2-azido-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid dimethyl ester (XV) (47 mg, 38%, less polar product) and (1S,2R,3R,5R,6S)-2-azido-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid dimethyl ester (XIV) (15 mg, 12%, more polar product) both as a colorless oil. (XV): ¹H-NMR (250 MHz, CDCl₃) δ 2.04-2.11 (2H, m), 2.20 (1H, bs), 2.36 (1H, dd, *J* = 6.6, 3.0 Hz), 2.45 (1H, dd, *J* = 13, 5.5 Hz), 2.56 (1H, t, *J* = 3.1 Hz), 3.70 (3H, s), 3.85 (3H, s); MS[ISP] 274 (M+NH₄⁺); (XIV): ¹H-NMR (250 MHz, CDCl₃) δ 1.84 (1H, t, *J* = 3.5 Hz), 2.07 (1H, dd, *J* = 6.5, 3.5 Hz), 2.16 (1H, dd, *J* = 12.5, 5.5 Hz), 2.27 (1H, dd, *J* = 6.5, 3.5 Hz), 2.33 (1H, s), 2.37 (1H, d, *J* = 12.5 Hz), 3.69 (3H, s), 3.90 (3H, s); MS[ISP] 274 (M+NH₄⁺);

Example 15

(1S,2R,3R,5R,6S)-2-Amino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-3)

A solution of (1S,2R,3R,5R,6S)-2-azido-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid dimethyl ester (XIV) (15 mg, 0.058 mmol) in HOAc (1 mL) and

H₂O (0.25 mL) was hydrogenated in the presence of Pd/C (5 mg, 10% Pd/C) at 23° C for 18 h. The catalyst was removed by filtration and the catalyst washed with water. After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (2 mL) for 4 h. The solution was cooled to 23° C and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (2 mL) and propylene oxide (0.5 mL) and refluxed for 15 min, whereupon the amino acid precipitated as white fluffy material. After cooling to 23° C, the product was filtered off, washed with ether, taken up in water and lyophilized to give (1S,2R,3R,5R,6S)-2-amino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-3) (9 mg, 76%) as a white solid. ¹H-NMR (250 MHz, D₂O) δ 1.45 (1H, t, *J* = 3.1 Hz), 1.71 (1H, m), 1.79 (1H, dd, *J* = 7, 3.0 Hz), 1.94 (1H, dd, *J* = 12.5, 5 Hz), 2.17 (1H, d, *J* = 12.6 Hz); MS[ISN] 201 [(M-H)⁻] mp >250° C; α_D²⁰ -0.94° (c = 0.32, H₂O).

Example 16

15 (1S,2R,3S,5R,6S)-2-Amino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-4)

A solution of (1S,2R,3S,5R,6S)-2-azido-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid dimethyl ester (XV) (47 mg, 0.183 mmol) in HOAc (2 mL) and H₂O (0.5 mL) was hydrogenated in the presence of Pd/C (9 mg, 10% Pd/C) at 23° C for 18 h. The catalyst was removed by filtration and the catalyst washed with water. After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (3 mL) for 4 h. The solution was cooled to 23° C and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (3 mL) and propylene oxide (1.5 mL) and refluxed for 15 min, whereupon the amino acid precipitated as white fluffy material. After cooling to 23° C, the product was filtered off, washed with ether, taken up in water and lyophilized to give (1S,2R,3S,5R,6S)-2-amino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-4) (32 mg, 86%) as a white solid. ¹H-NMR (250 MHz, D₂O) δ 2.05 (1H, d, *J* = 15 Hz), 2.14 (2H, m), 2.23 (1H, dd, *J* = 7, 3.5 Hz), 2.60 (1H, dd, *J* = 15, 5 Hz); MS[ISN] 201 [(M-H)⁻]; mp >250° C; α_D²⁰ +31.26° (c = 0.12, H₂O).

30

Example 17

(1S,2R,5R,6S)-2-Acetylamino-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XVI)

- A solution of (1S,2R,5R,6S)-2-Azido-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (IX) (974 mg, 2.84 mmol) in thioacetic acid (6.5 mL) was stirred at 70° C for 2 d. The reaction mixture was concentrated in vacuum and subjected to silica gel column chromatography with hexane/ethyl acetate 3:2 -> 1:1 to yield
- 5 (1S,2R,5R,6S)-2-acetylamino-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XVI) (726 mg, 71%) as a pink oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.25 (3H, t, *J* = 7.1 Hz), 1.63 (1H, t, *J* = 3.5 Hz), 2.05 (3H, s), 2.32 (1H, ddd, *J* = 7, 6, 3.5 Hz), 2.54 (1H, d, *J* = 19 Hz), 2.90 (1H, dd, *J* = 19, 6 Hz), 3.06 (1H, dd, *J* = 7, 3.5 Hz), 4.12 (2H, m), 5.16 (1H, d, *J* = 12 Hz), 5.25 (1H, d, *J* = 12 Hz), 7.24-7.35 (5H, m); MS[ISP] 360
- 10 (M+H⁺); α_D²⁰ +44.33° (c = 0.97, CHCl₃).

Example 18

- (1S,2R,3R,5R,6S)-2-Acetylamino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XVII) and (1S,2R,3S,5R,6S)-2-Acetylamino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic 2-benzyl ester 6-ethyl ester (XVIII)
- 15

- To a solution of (1S,2R,5R,6S)-2-acetylamino-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic 2-benzyl ester 6-ethyl ester (XVI) (77 mg, 0.214 mmol) in EtOH (2 mL) and THF (1 mL) was added LiBH₄ (5 mg, 0.23 mmol) at -50° C and the mixture was stirred at -50° C for 45 min. The reaction was quenched by addition of 1N HCl (ca. 0.5 mL),
- 20 warming up to 23° C and stirring for 10 min. After dilution with ethyl acetate and extraction with sat. NaHCO₃-sol., brine and drying over MgSO₄, the crude product was purified by silica gel column chromatography with toluene/acetone 3:1 to give
- (1S,2R,3R,5R,6S)-2-acetylamino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XVII) (33 mg, 43%, less polar product) as a colorless oil and
- 25 (1S,2R,3S,5R,6S)-2-acetylamino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XVIII) (35 mg, 45%, more polar product) as a white solid.
- (XVII): ¹H-NMR (250 MHz, CDCl₃) δ 1.26 (3H, t, *J* = 7.2 Hz), 1.76 (1H, t, *J* = 3.5 Hz), 2.07 (1H, m), 2.10 (3H, s), 2.16 (1H, dd, *J* = 6.1, 3.0 Hz), 2.30-2.41 (3H, m), 3.97-4.13 (3H, m), 5.24 (2H, bs), 6.19 (1H, bs), 7.26-7.38 (5H, m); MS[ISP] 362 (M+H⁺); α_D²⁰ -
- 30 11.24° (c = 1.18, CHCl₃). (XVIII): ¹H-NMR (250 MHz, CDCl₃) δ 1.25 (3H, t, *J* = 7.1 Hz), 1.98 (1H, m), 2.05-2.11 (1H, m), 2.06 (3H, s), 2.18 (1H, t, *J* = 3.5 Hz), 2.27-2.41 (2H, m), 2.59 (1H, ddd, *J* = 13, 7, 5 Hz), 4.12 (2H, m), 4.68 (1H, dd, *J* = 7, 5 Hz), 5.17 (2H, s), 6.30 (1H, bs), 7.26-7.40 (5H, m); MS[ISP] 362 (M+H⁺); mp 145-147° C; α_D²⁰ +42.44° (c = 1.01, CHCl₃).

Example 19

(1S,2R,3R,5R,6S)-2-Acetylamino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-
dicarboxylic acid 2-benzyl ester 6-ethyl ester (XIX) and (1S,2R,3S,5R,6S)-2-Acetylamino-
5 3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic 2-benzyl ester 6-ethyl ester
(XX)

To a solution of (1S,2R,5R,6S)-2-acetylamino-3-oxo-bicyclo[3.1.0]hexane-2,6-
dicarboxylic 2-benzyl ester 6-ethyl ester (XVI) (105 mg, 0.292 mmol) in EtOH (1.9 mL)
and THF (1.0 mL) was added NaBD₄ (24.5 mg, 0.584 mmol) at -50° C and the mixture
10 was stirred at -50° C for 90 min. The reaction was quenched by addition of HOAc (ca. 0.5
mL), warming up to 23° C and stirring for 10 min. After dilution with ethyl acetate, 1N
HCl was added, followed by washings with sat. NaHCO₃-sol., brine and drying over
MgSO₄. After removal of the solvent in vacuum the crude product was purified by silica
gel column chromatography with toluene/acetone 3:1 to give (1S,2R,3R,5R,6S)-2-
15 acetylamino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl
ester 6-ethyl ester (XIX) (45 mg, 43%, less polar product) as a colorless oil and
(1S,2R,3S,5R,6S)-2-acetylamino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-
dicarboxylic acid 2-benzyl ester 6-ethyl ester (XX) (50 mg, 47%, more polar product) as a
white solid. (XIX): ¹H-NMR (250 MHz, CDCl₃) δ 1.26 (3H, t, J = 7.2 Hz), 1.98 (1H, m),
20 1.74 (1H, t, J = 3.5 Hz), 2.04-2.10 (1H, m), 2.09 (3H, s), 2.17 (1H, dd, J = 7, 2.9 Hz),
2.35-2.40 (3H, m), 4.10 (2H, m), 5.24 (2H, s), 6.33 (1H, bs), 7.26-7.37 (5H, m); MS[ISP]
363 (M+H⁺); (XX): ¹H-NMR (250 MHz, CDCl₃) δ 1.24 (3H, t, J = 7.1 Hz), 1.97 (1H, ddd,
J = 8, 6.5, 3 Hz), 2.04 (3H, s), 2.06 (1H, dd, J = 13.5, 3.7 Hz), 2.17 (1H, t, J = 3.1 Hz),
2.32 (1H, dd, J = 6.6, 3.0 Hz), 2.56 (1H, dd, J = 13.5, 5 Hz), 2.58 (1H, s), 4.09 (2H, m),
25 5.23 (2H, s), 6.42 (1H, bs), 7.28-7.40 (5H, m); MS[ISP] 363 (M+H⁺);

Example 20

(1S,2R,3R,5R,6S)-2-Acetylamino-3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-
dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXI) and (1S,2R,3S,5R,6S)-2-Acetylamino-
30 3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-dicarboxylic 2-benzyl ester 6-ethyl ester
(XXII)

Radiochemical samples were counted in a wallac WinSpectral 1414 Liquid Scintillation
Counter using OptiPhase 'HiSafe'3 as scintillation cocktail.

n-Butyl lithium (200 μ L, 0.312 mmol, 1.56M in n-hexane) and N,N,N',N'-tetramethylethylenediamine (52 μ L, 0.347 mmol) were transferred into a 7.5 mL-two-necked-flask mounted to the tritiation apparatus under argon. The stopcock of the side neck was closed and the mixture was stirred under an atmosphere of tritium gas for 2 h 43 min, while the tritium gas pressure dropped from 646 mbar to 559 mbar. Excess tritium gas was reabsorbed onto the uranium bed and the volatile components were lyophilized off. The residue was dried at 10^{-3} mbar for about 5 min. The flask was filled with dry nitrogen and the LiT was suspended in THF (250 μ L), which was added by syringe through the silicon septum (Hamilton # 76005) of the side neck. Then trimethylborate (35 μ L, 0.314 mmol) was added and the mixture was stirred for 10 min. The two-necked-flask was disconnected from the tritiation apparatus and equipped with a balloon filled with argon. (1S,2R,5R,6S)-2-acetylamino-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic 2-benzyl ester 6-ethyl ester (XVI) (118.7 mg, 0.33 mmol) in THF (250 μ L) was added at -60° C. After stirring for 30 min the reaction was quenched by adding 1N HCl (0.31 mL). The reaction mixture was partitioned between EtOAc and H₂O. The organic phase was washed with saline (1 \times) and dried over Na₂SO₄. The total ³H-activity of the crude product was 5.18 Ci. Column chromatography using 15g of Lichroprep Si60 25-40 μ m (Merck Art.1.09390) with toluene/acetone 5:1 afforded 1.152 Ci of (1S,2R,3R,5R,6S)-2-acetylamino-3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXI) with 99% radiochemical purity according to TLC (toluene/acetone 3:1). The specific activity was 23.9 Ci/mmol according to mass spectrometry. In addition about 2 Ci of (1S,2R,3S,5R,6S)-2-acetylamino-3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXII) were isolated.

25

Example 21

(1S,2R,3R,5R,6S)-2-Amino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-3)

(1S,2R,3R,5R,6S)-2-Acetylamino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XIX) (15 mg, 0.058 mmol) was refluxed in 10% HCl (3.7 mL) for 6 h. The solution was cooled to 23° C and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (3 mL) and propylene oxide (1.6 mL) and refluxed for 15 min, whereupon the amino acid precipitated as white fluffy material. After cooling to 23° C, the product was filtered off, washed with ether, taken up in water and lyophilized to give (1S,2R,3R,5R,6S)-2-amino-3-deutero-3-hydroxy-

bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-3) (12 mg, 71%) as a white solid. Analytical data the same as in example 15.

Example 22

5 (1S,2R,3R,5R,6S)-2-Amino-3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-5)

(1S,2R,3R,5R,6S)-2-Acetylamino-3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXI) (576 mCi) was refluxed in 10% HCl (5 mL) for 6 h. The reaction mixture was diluted with H₂O (5 mL) and applied onto a
10 Dowex 50WX8 100-200 mesh cation exchange column (7.5 × 70 mm). The column was rinsed with H₂O (20 mL). Elution with 2N NH₄OH (20 mL) afforded (1S,2R,3R,5R,6S)-2-amino-3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-5) (280 mCi). The radiochemical purity was 91.7% according to TLC (n-BuOH/HOAc/H₂O 3:1:1). The crude product (56 mCi) was purified by HPLC on a μ Bondapak C18 column (3.9 × 300 mm)
15 using H₂O/acetonitrile 95:5 (v/v) as mobile phase with a flow rate of 0.8 mL/min and UV-detection at 220 nm. The total ³H-activity obtained was 43 mCi and the radiochemical purity was 98.1% according to TLC (n-BuOH/HOAc/H₂O 3:1:1).

Example 23

20 (1S,2R,3R,5R,6S)-2-Azido-3-methoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-1)

A solution of (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII-1) (345 mg, 1.0 mmol), 2,6-di-*tert*-butylpyridine (1.35 mL, 6.0 mmol) and methyl triflate (0.55 mL, 5.0 mmol) in DCM (2
25 mL) was stirred at 23° C for 4 d. The reaction mixture was poured on ice, acidified with 1 N HCl and extracted with ether. After washing with sat. NaHCO₃-sol., brine and drying over MgSO₄, the crude product was purified by silica gel column chromatography with hexane/EtOAc 4:1 to give (1S,2R,3R,5R,6S)-2-azido-3-methoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-1) (226 mg, 63%) as a yellow oil.
30 ¹H-NMR (250 MHz, CDCl₃) δ 1.26 (3H, t, *J* = 7.1 Hz), 1.77 (1H, t, *J* = 3.5 Hz), 2.05-2.18 (2H, m), 2.25 (1H, dd, *J* = 7, 3.5 Hz), 2.34 (1H, dd, *J* = 12, 7 Hz), 3.28 (3H, s), 3.42 (1H, bt, *J* = 8 Hz), 4.12 (2H, q, *J* = 7.1 Hz), 5.25 (1H, d, *J* = 12 Hz), 5.34 (1H, d, *J* = 12 Hz), 7.30-7.40 (5H, m); MS[EI] 258 [(M-CO₂Et-N₂)⁺]; α_D^{20} -48.02° (*c* = 1.11, CHCl₃).

Example 24

(1S,2R,3R,5R,6S)-3-Allyloxy-2-azido-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-2)

To a solution of (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII) (200 mg, 0.58 mmol) and allyl 2,2,2-trichloroacetimidate (0.18 mL, 1.16 mmol) in cyclohexane (0.7 mL) was added TfOH (29 μ L), whereupon the solution became hot and stirring was continued at 23° C for 1 h. The reaction mixture was poured on ice, diluted with ether, washed with sat. NaHCO₃-sol., brine and dried over MgSO₄. The crude product was purified by silica gel column chromatography with hexane/EtOAc 4:1 to give (1S,2R,3R,5R,6S)-3-allyloxy-2-azido-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-2) (31 mg, 14%) as a yellow oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.26 (3H, t, J = 7.1 Hz), 1.76 (1H, t, J = 3.5 Hz), 2.03-2.26 (3H, m), 2.32 (1H, dd, J = 12, 7 Hz), 3.58 (1H, dd, J = 9, 7 Hz), 3.92 (1H, m), 4.02 (1H, m), 4.13 (2H, q, J = 7.1 Hz), 5.10-5.22 (2H, m), 5.25 (1H, d, J = 12 Hz), 5.34 (1H, d, J = 12 Hz), 5.75 (1H, m), 7.30-7.42 (5H, m); MS[EI] 284 [(M-CO₂Et-N₂)⁺]; α_D^{20} -17.85° (c = 0.50, CHCl₃).

Example 25

(1S,2R,3R,5R,6S)-2-Azido-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-3)

To a solution of (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII) (305 mg, 0.88 mmol) and benzyl 2,2,2-trichloroacetimidate (0.2 mL, 1.06 mmol) in cyclohexane (2.4 mL) and DCM (1.2 mL) was added TfOH (0.05 mL), whereupon the solution became hot and stirring was continued at 23° C for 5 h. The reaction mixture was poured on ice, diluted with ether, washed with sat. NaHCO₃-sol., brine and dried over MgSO₄. The crude product was purified by silica gel column chromatography with hexane/EtOAc 4:1 to give (1S,2R,3R,5R,6S)-2-azido-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-3) (102 mg, 27%) as a yellow oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.25 (3H, t, J = 7.1 Hz), 1.75 (1H, t, J = 3.5 Hz), 2.06 (1H, m), 2.17-2.31 (3H, m), 3.60 (1H, bt, J = 8 Hz), 4.12 (2H, q, J = 7.1 Hz), 4.42 (1H, d, J = 12 Hz), 4.57 (1H, d, J = 12 Hz), 5.23 (1H, d, J = 12 Hz), 5.34 (1H, d, J = 12 Hz), 7.15-7.42 (10H, m); MS[ISP] 408 [(M+H-N₂)⁺]; α_D^{20} +2.03° (c = 0.99, CHCl₃).

Example 26

(1S,2R,3R,5R,6S)-3-Allyloxy-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIV-2)

To a solution of (1S,2R,3R,5R,6S)-3-allyloxy-2-azido-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-2) (101 mg, 0.262 mmol) in THF (3.9 mL) and H₂O (0.4 mL) was added Me₃P (0.29 mL, 0.29 mmol, 1M sol. in THF) and stirring was continued at 23° C for 3 h. The reaction mixture was diluted with ether, washed with sat. NaHCO₃-sol., brine and dried over Na₂SO₄. The crude product was purified by silica gel column chromatography with hexane/EtOAc 1:1 (+ small amount of Et₃N) to give (1S,2R,3R,5R,6S)-3-allyloxy-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIV-2) (47 mg, 50%) as a light brown oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.24 (3H, t, J = 7.1 Hz), 1.76 (1H, t, J = 3.5 Hz), 1.93 (2H, bs), 1.99 (1H, m), 2.07-2.18 (2H, m), 2.31 (1H, dd, J = 12.5, 7 Hz), 3.45 (1H, bt, J = 8 Hz), 3.93 (2H, bd, J = 6 Hz), 4.12 (2H, q, J = 7.1 Hz), 5.10-5.35 (4H, m), 5.78 (1H, m), 7.28-7.38 (5H, m); MS[ISP] 360 [(M+H)⁺]; α_D²⁰ -10.67° (c = 1.00, CHCl₃).

Example 27

(1S,2R,3R,5R,6S)-2-Amino-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIV-3)

To a solution of (1S,2R,3R,5R,6S)-2-azido-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-3) (120 mg, 0.276 mmol) in THF (4 mL) and H₂O (0.4 mL) was added Me₃P (0.30 mL, 0.30 mmol, 1M sol. in THF) and stirring was continued at 23° C for 4 h. The reaction mixture was diluted with ether, washed with sat. NaHCO₃-sol., brine and dried over Na₂SO₄. The crude product was purified by silica gel column chromatography with hexane/EtOAc 1:1 (+ small amount of Et₃N) to give (1S,2R,3R,5R,6S)-2-amino-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIV-3) (58 mg, 51%) as a yellow oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.23 (3H, t, J = 7.1 Hz), 1.72 (1H, t, J = 3.5 Hz), 1.93 (2H, bs), 2.00 (1H, m), 2.10 (1H, dd, J = 7, 3.5 Hz), 2.19 (1H, m), 2.27 (1H, dd, J = 12, 7 Hz), 3.46 (1H, dd, J = 8, 7 Hz), 4.10 (2H, q, J = 7.1 Hz), 4.47 (2H, s), 5.20 (1H, d, J = 12 Hz), 5.30 (1H, d, J = 12 Hz), 7.18-7.42 (10H, m); MS[ISP] 410 [(M+H)⁺]; α_D²⁰ +3.54° (c = 0.90, CHCl₃).

Example 28

(1S,2R,3R,5R,6S)-2-Amino-3-methoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-7)

A solution of (1S,2R,3R,5R,6S)-2-azido-3-methoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-1) (214 mg, 0.56 mmol) in HOAc (8 mL) and H₂O (2 mL) was hydrogenated in the presence of Pd/C (35 mg, 10% Pd/C) at 23° C for 18 h. The catalyst was removed by filtration, the filter cake washed with 50% aqueous acetic acid. After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (15 mL) for 4 h. The solution was cooled to 23° C, filtered, washed with water and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (10 mL) and propylene oxide (5.6 mL) and refluxed for 15 min, whereupon the amino acid precipitated. After cooling to 23° C, the product was filtered off, washed with ether and dried to give (1S,2R,3R,5R,6S)-2-amino-3-methoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-7) (100 mg, 74%) as a white solid. ¹H-NMR (250 MHz, D₂O) δ 1.86 (1H, t, *J* = 3.5 Hz), 2.04-2.23 (3H, m), 2.50 (1H, dd, *J* = 12, 7 Hz), 3.29 (3H, s), 3.62 (1H, bt, *J* = 8 Hz); MS[ISN] 214 [(M-H)⁺]; mp >250° C; α_D²⁰ -13.84° (c = 1.01, H₂O).

Example 29

(1S,2R,3R,5R,6S)-2-Amino-3-propoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-8)

A solution of (1S,2R,3R,5R,6S)-3-allyloxy-2-azido-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIII-2) (28 mg, 0.073 mmol) in HOAc (0.75 mL) and H₂O (0.25 mL) was hydrogenated in the presence of Pd/C (3 mg, 10% Pd/C) at 23° C for 18 h. The catalyst was removed by filtration, the filter cake washed with 50% aqueous ethanol. After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (1.25 mL) for 4 h. The solution was cooled to 23° C and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (1 mL) and propylene oxide (0.5 mL) and refluxed for 10 min, whereupon the amino acid precipitated. After cooling to 23° C, the product was filtered off, washed with ether and dried to give (1S,2R,3R,5R,6S)-2-amino-3-propoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-8) (13 mg, 72%) as a white solid. ¹H-NMR (250 MHz, D₂O) δ 0.81 (1H, t, *J* = 7.5 Hz), 1.48 (2H, m), 1.84 (1H, m), 2.16 (3H, m), 2.26 (1H, dd, *J* = 12, 7 Hz), 3.43 (2H, m), 3.73 (1H, bt, *J* = 8 Hz); MS[ISN] 242 [(M-H)⁺]; mp >250° C; α_D²⁰ -3.72° (c = 0.19, H₂O).

Example 30

(1S,2R,3R,5R,6S)-3-Allyloxy-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-9)

A solution of (1S,2R,3R,5R,6S)-3-allyloxy-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIV-2) (47 mg, 0.131 mmol) and LiOH•H₂O (15 mg, 0.357 mmol) in THF (4 mL), H₂O (2 mL) and MeOH (0.4 mL) was stirred at 23° C for 36 h. The solution was acidified with conc. HCl and evaporated to dryness. The remaining pale yellow solid was suspended in EtOH, filtered, washed with more EtOH and the filtrate was evaporated to dryness. The residue was dissolved in EtOH (1 mL) and propylene oxide (1 mL) and refluxed for 3 min, whereupon the amino acid precipitated. After cooling to 23° C, the product was filtered off, washed with ether and dried to give (1S,2R,3R,5R,6S)-3-allyloxy-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-9) (19 mg, 59%) as a white solid. ¹H-NMR (250 MHz, D₂O) δ 1.48 (1H, t, *J* = 3.5 Hz), 1.78 (2H, m), 1.98 (1H, ddd, *J* = 12.5, 8, 3.5 Hz), 2.27 (1H, dd, *J* = 12.5, 7 Hz), 3.44 (1H, bt, *J* = 7.5 Hz), 3.97 (2H, m), 5.13-5.26 (2H, m), 5.82 (1H, ddt, *J* = 18, 11, 6 Hz); MS[ISN] 240 [(M-H)⁻]; mp >250° C; α_D²⁰ -10.62° (c = 0.40, H₂O).

Example 31

(1S,2R,3R,5R,6S)-2-Amino-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-10)

A solution of (1S,2R,3R,5R,6S)-2-amino-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXIV-3) (50 mg, 0.122 mmol) and LiOH•H₂O (13 mg, 0.30 mmol) in THF (4 mL), H₂O (2 mL) and MeOH (0.4 mL) was stirred at 23° C for 36 h. The solution was acidified with conc. HCl and evaporated to dryness. The remaining pale yellow solid was suspended in EtOH, filtered, washed with more EtOH and the filtrate was evaporated to dryness. The residue was dissolved in EtOH (1 mL) and propylene oxide (1 mL) and refluxed for 3 min, whereupon the amino acid precipitated. After cooling to 23° C, the product was filtered off, washed with ether and dried to give (1S,2R,3R,5R,6S)-2-amino-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-10) (26 mg, 72%) as a white solid. ¹H-NMR (250 MHz, D₂O) δ 1.57 (1H, t, *J* = 3.5 Hz), 1.97 (2H, m), 2.13 (1H, ddd, *J* = 12.5, 8, 3.5 Hz), 2.35 (1H, dd, *J* = 12.5, 7 Hz), 3.73 (1H, dd, *J* = 8, 7 Hz), 4.51 (2H, s), 7.36 (5H, bm); MS[ISN] 290 [(M-H)⁻]; mp >250° C; α_D²⁰ -5.69° (c = 0.25, H₂O).

Example 32

(1S,2R,3R,5R,6S)-2-Azido-3-trifluoromethanesulfonyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXV)

To a solution of (1S,2R,3R,5R,6S)-2-azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (VII-1) (518 mg, 1.5 mmol) and pyridine (0.36 mL, 4.5 mmol) in DCM (19 mL) at -78° C was added trifluoromethanesulfonic anhydride (0.37 mL, 2.25 mmol) in DCM (1.4 mL) and the mixture was allowed to reach 0° C. The reaction mixture was diluted with ether, poured on ice, extracted with sat. CuSO₄-sol. and brine, followed by drying over Na₂SO₄. After removal of the solvent in vacuum, the crude product was purified by silica gel column chromatography with hexane/EtOAc 4:1 to give (1S,2R,3R,5R,6S)-2-azido-3-trifluoromethanesulfonyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXV) (574 mg, 86%) as a colorless oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.27 (3H, t, *J* = 7.1 Hz), 1.84 (1H, t, *J* = 3.5 Hz), 2.17 (1H, m), 2.34 (1H, dd, *J* = 7, 3.5 Hz), 2.53 (2H, m), 4.15 (2H, q, *J* = 7.1 Hz), 4.58 (1H, bt, *J* = 8 Hz), 5.26 (1H, d, *J* = 12 Hz), 5.41 (1H, d, *J* = 12 Hz), 7.39 (5H, s); MS[ISP] 495 (M+NH₄⁺); α_D²⁰ -17.30° (c = 1.09, CHCl₃).

Example 33

(1S,2R,5R,6S)-2-Azido-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVI-1)

To a solution of (1S,2R,3R,5R,6S)-2-azido-3-trifluoromethanesulfonyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXV) (1.36 g, 2.85 mmol) in THF (5.7 mL) was added DBU (0.47 mL, 3.13 mmol) and the mixture was stirred at 50° C for 3 h. After dilution with ethyl acetate, the solution was washed with 1N HCl-sol., sat. NaHCO₃-sol. and brine, followed by drying over MgSO₄. Removal of the solvent in vacuum left the crude (1S,2R,5R,6S)-2-azido-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVI) (566 mg, 61%) as a brownish oil, sufficiently pure for further transformations. ¹H-NMR (250 MHz, CDCl₃) δ 1.27 (3H, t, *J* = 7.1 Hz), 1.76 (1H, t, *J* = 3.5 Hz), 2.59 (1H, dt, *J* = 7, 3.5 Hz), 2.67 (1H, ddd, *J* = 7, 3.5, 2 Hz), 4.14 (2H, q, *J* = 7.1 Hz), 5.27 (2H, s), 5.53 (1H, d, *J* = 5.5 Hz), 6.25 (1H, dd, *J* = 5.5, 2 Hz), 7.37 (5H, s); MS[ISP] 345 (M+NH₄⁺); α_D²⁰ -183.54° (c = 1.08, CHCl₃).

Example 34

(1S,2R,5R,6S)-2-Amino-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVII)

To a solution of (1S,2R,5R,6S)-2-azido-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVI) (66 mg, 0.202 mmol) in THF (4.5 mL) and H₂O (0.5 mL) was added polymer supported PPh₃ (130 mg, 0.404 mmol, 3.1 mmol/g) and the mixture was stirred at 60° C for 19 h. The resin was filtered off and washed with DCM. Removal of the solvent in vacuum left a yellow oil which was purified by silica gel column chromatography with hexane/ethyl acetate 1:3 to give (1S,2R,5R,6S)-2-amino-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVII) (19 mg, 31%) as a colorless oil. ¹H-NMR (250 MHz, CDCl₃) δ 1.25 (3H, t, *J* = 7.2 Hz), 1.55 (1H, t, *J* = 2.9 Hz), 1.78 (2H, bs), 2.53 (2H, m), 4.12 (2H, q, *J* = 7.2 Hz), 5.21 (2H, s), 5.37 (1H, d, *J* = 5.3 Hz), 6.07 (1H, dd, *J* = 5.3, 2 Hz), 7.35 (5H, s); MS[ISP] 302 (M+H⁺); α_D²⁰ - 322.98 (c = 0.77, CHCl₃).

Example 35

(1S,2R,5R,6S)-2-Amino-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid (I-10)

A solution of (1S,2R,5R,6S)-2-amino-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVII) (19 mg, 0.063 mmol) and LiOH•H₂O (8 mg, 0.19 mmol) in THF (2 mL), H₂O (0.5 mL) and MeOH (0.2 mL) was stirred at 23° C for 10 h. The solution was acidified with conc. HCl and evaporated to dryness. The remaining pale yellow solid was suspended in EtOH, filtered, washed with more EtOH and the filtrate was evaporated to dryness. The residue was dissolved in EtOH (1 mL) and propylene oxide (1 mL) and refluxed for 3 min, whereupon the amino acid precipitated. After cooling to 23° C, the product was filtered off, washed with ether and dried to give (1S,2R,5R,6S)-2-amino-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid (I-10) (11 mg, 95%) as a white solid. ¹H-NMR (250 MHz, D₂O) δ 1.59 (1H, t, *J* = 3 Hz), 2.32 (1H, m), 2.60 (1H, m), 5.44 (1H, d, *J* = 5.5 Hz), 6.37 (1H, dd, *J* = 5.5, 2 Hz); MS[ISN] 182 [(M-H)⁻]; mp >250° C;

Example 36

(1S,2R,5R,6S)-2-Amino-3,4-ditritio-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid (I-B)

Radiochemical samples were counted in a Berthold BF 5020 liquid scintillation counter using Safetron-150 as scintillation cocktail.

A solution of (1S,2R,5R,6S)-2-amino-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid (I-10) (4.4 mg, 20 μ mol) in EtOH (0.5 mL) and H₂O (0.5 mL) was stirred in the presence of Pd/C (1 mg, 10% Pd on activated carbon) under an atmosphere of tritium gas for 1 h at 23° C. Excess tritium gas was reabsorbed onto the uranium bed and the solvent was lyophilized off. The residue was thrice taken up in EtOH (0.5 mL) and H₂O (0.5 mL), stirred briefly and lyophilized to ensure complete removal of labile bound tritium. The residue was suspended in EtOH/H₂O 1:1 and the catalyst was removed by filtration through a 0.45 μ m Millex-HA cartridge. The total activity of the crude product was 718 mCi. The radiochemical purity was 94.3% according to TLC [n-BuOH/HOAc/H₂O 3:1:1]. Part of the crude product (72 mCi) was purified by HPLC on a Nucleosil C8 column (5 μ m, 4 \times 250 mm), using a 25 mM KH₂PO₄-sol. as mobile phase with a flow rate of 0.5 mL/min and UV-detection at 220 nm. In order to remove the buffer salt from the mobile phase the sample was applied onto a SP-Sephadex cation exchange column (10 \times 115 mm, H⁺-form). The column was rinsed with H₂O (20 mL) and the compound was eluted with 2N NH₄OH while 5 min-fractions were collected. Fractions 6 and 7 containing ³H-activity were pooled and the volume was reduced to ca. 2 mL by evaporation at ca. 25 mbar. The sample was diluted to 20 ml with EtOH/H₂O 1:1. The total activity was 37.5 mCi. The radiochemical purity was 98.3% according to TLC [n-BuOH/HOAc/H₂O 3:1:1]. The specific activity determined by mass spectrometry was 35 Ci/mmol.

Example 37

(1S,2R,3S,5R,6S)-2,3-Diazido-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVIII-1)

To a solution of (1S,2R,3R,5R,6S)-2-azido-3-trifluoromethanesulfonyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXV) (200 mg, 0.48 mmol) in DMF (0.8 mL) was added NaN₃ (187 mg, 2.88 mmol) and the mixture was stirred at 80° C for 1 h. After cooling to 23° C, the reaction was poured onto ice, extracted with ethyl acetate, washed with brine and dried over MgSO₄. Removal of the solvent in vacuum left the crude (1S,2R,3S,5R,6S)-2-diazido-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVIII), contaminated with ca. 12% of (1S,2R,5R,6S)-2-azido-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVI), which was removed by reacting the mixture with OsO₄ (3 drops of a 2.5% sol. in t-BuOH), NMO (18 mg, 0.162 mmol) in acetone (1.5 mL) and H₂O (3 mL) at 23° C for 24 h. After quenching with sodium sulfite (ca. 150 mg) the reaction was poured

onto ice, extracted with EtOAc (3 × 50 mL) and dried over MgSO₄. After removal of the solvent in vacuum, the residue was purified by silica gel column chromatography with hexane/ethyl acetate 4:1 to give (1S,2R,3S,5R,6S)-2-diazo-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVIII) (81 mg, 49%) as a colorless oil.

- 5 ¹H-NMR (250 MHz, CDCl₃) δ 1.27 (3H, t, *J* = 7.2 Hz), 1.97 (1H, m), 2.02 (1H, d, *J* = 15.2 Hz), 2.32-2.40 (2H, m), 2.48 (1H, ddd, *J* = 15, 7.5, 5 Hz), 4.14 (2H, q, *J* = 7.2 Hz), 4.20 (1H, d, *J* = 7.4 Hz), 5.28 (2H, s), 7.39 (5H, s); MS[ISP] 388 (M+NH₄⁺); α_D²⁰ -56.71° (c = 1.11, CHCl₃).

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Example 38

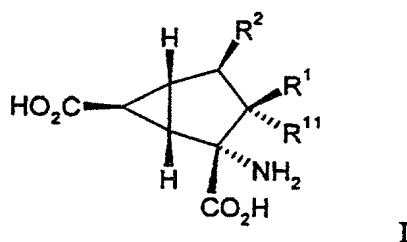
(1S,2R,3S,5R,6S)-2,3-Diamino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-12)

- A solution of (1S,2R,3S,5R,6S)-2-diazo-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (XXVIII-1) (45 mg, 0.122 mmol) in HOAc (3.8 mL) and H₂O (0.95 mL) was hydrogenated in the presence of Pd/C (12 mg, 10% Pd/C) at 23° C for
15 18 h. The catalyst was removed by filtration, the filter cake washed with water. After removal of the solvent in vacuum, the brown residue was refluxed in 10% HCl (8.9 mL) for 4 h. The solution was cooled to 23° C, filtered, washed with water and evaporated to dryness. The remaining beige solid was dissolved in EtOH (7.1 mL) and propylene oxide (3.8 mL) and refluxed for 15 min, whereupon the amino acid precipitated. After cooling to
20 23° C, the product was filtered off, washed with ether and dried to give (1S,2R,3S,5R,6S)-2,3-diamino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (I-12) (19 mg, 79%) as a beige solid. ¹H-NMR (250 MHz, D₂O) δ 1.80 (1H, t, *J* = 3 Hz), 2.00 (1H, dd, *J* = 15, 5 Hz), 2.10 (1H, m), 2.15 (1H, dd, *J* = 5, 3 Hz), 2.82 (1H, ddd, *J* = 15, 10, 5 Hz), 4.07 (1H, dd, *J* = 10, 5 Hz); MS[ISN] 199 [(M-H)⁻]; mp >250° C; α_D²⁰ +9.50° (c = 0.29, H₂O).

25

Claims

1. A process for the preparation of compounds of formula



5 wherein

R^1 is hydroxy, lower alkoxy, lower alkenyloxy, benzyloxy, hydrogen, deuterium or tritium,

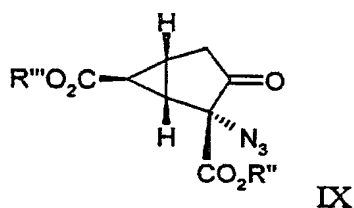
R^{11} is hydrogen, deuterium or optionally tritium, hydroxy or amino and

R^2 is hydrogen or optionally tritium or

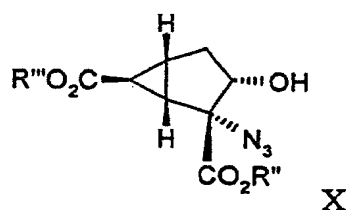
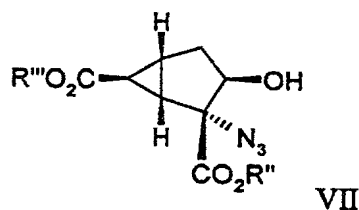
10 R^1 and R^2 form a bond

which process comprises

a) reducing the carbonyl group of a compound of formula



to a hydroxy group of a compound of formula VII or formula X



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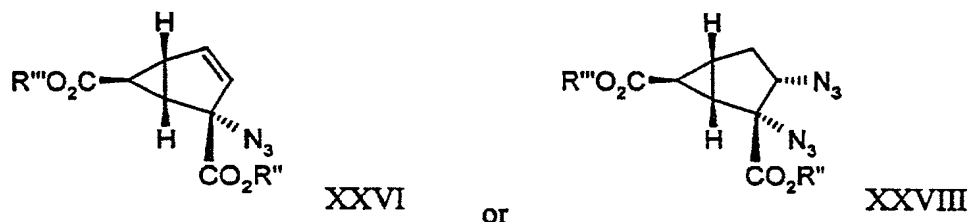
wherein R''' and R'' are independently from each other benzyl or lower alkyl and, if desired,

transforming the hydroxy group of a compound of formula VII or X into a leaving group and

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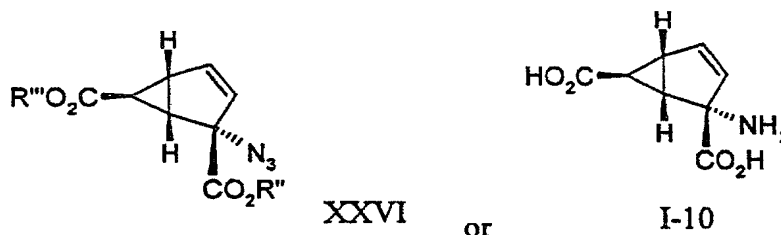
1.) eliminating this group to obtain a compound of formula XXVI or

2.) substituting said leaving group with azide to a compound of formula XXVIII



wherein R''' and R'' are independently from each other benzyl or lower alkyl and, if desired,

- 5 b) alkylating, alkenylating or benzylating a compound of formula VII
- and
- c) reducing the azide group(s) into (an) amino group(s),
- and
- d) hydrolizing the ester groups, and, if desired,
- 10 e) hydrogenating the double bond in compounds of formulae

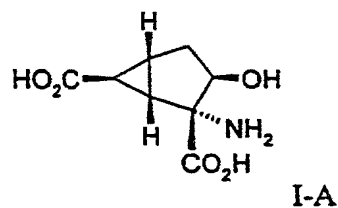


wherein R''' and R'' are independently from each other benzyl or lower alkyl with tritium-gas.

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2. A process in accordance with claim 1, wherein the leaving group is the trifluoromethyl sulfonic ester group.

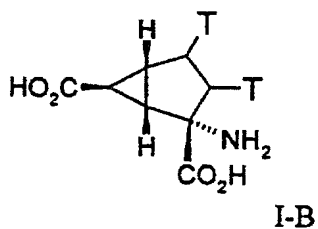
3. A compound of formula



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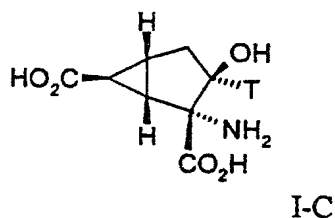
in accordance with formula I in claim 1.

4. A compound of formula



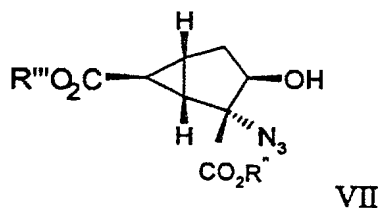
wherein T is tritium in accordance with formula I in claim 1.

5. A compound of formula



wherein T is tritium in accordance with formula I in claim 1.

6. A compound of formula



wherein R'' and R''' are independently from each other benzyl or lower alkyl.

7. A compound of formulae I-A, I-B or I-C as prepared by a process described in claim 1 or by an equivalent process.

8. The use of compounds of formulae I-B and I-C in accordance with claims 4 and 5 as a radio ligand in a binding assay in order to screen chemical libraries for new structures.

9. The use of a compound of formula I-A in accordance with claim 3 for proof of concept studies in animal models of human diseases.

10. The use of a compound of formula I-A in accordance with claim 3 in the control or prevention of illnesses.

11. The use of a compound of formula I-A in accordance with claim 3 for the manufacture of medicaments for the control or prevention of acute and chronic neurological conditions and psychiatric disorders.

5 12. The use of a compound of formula VII in accordance with claim 6 as intermediate for the preparation of compounds of formula I in accordance with claim 1.

13. A compound of formula I-A in accordance with claim 3 for use in therapy, particularly in the control or prevention of illnesses.

14. The invention as hereinbefore described.

15. Any novel feature or combination of features described herein.



Application No: GB 9920579.1
Claims searched: 1-15

Examiner: Dr William Thomson
Date of search: 22 November 1999

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:
UK Cl (Ed.Q): C2C (CLR)
Int Cl (Ed.6):
Other: ONLINE: CAS-ONLINE

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	EP 0774455A1 (ELI LILLY & CO) See whole document, in particular page 2, line 34 - page 3, line 20 and page 9, line 54 - page 10, line 21	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.